Fish Processing Method

Standard Operating Procedure SOP No. HC 523A.SOP

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Fish Processing Method

The following aging, compositing, and grinding method was used for fish collected for the Lake Michigan Mass Balance Study.

Fish were collected for the Lake Michigan Mass Balance study during the spring, summer, and fall of 1994, and spring and fall of 1995 from Sturgeon Bay, Port Washington, and Saugatuck on Lake Michigan. Information on the species, and number of fish caught is shown in Table 1. Coho shown in Table 1 were collected along varying locations each season (depending on migration) in 1994 and in 1995 collection occurred only during the spring and fall.

Table 1. Species, Seasons, and Number of Fish Collected for the Lake Michigan Mass Balance Study.

Biota Sampled	Spring 94	Summer 94	Fall 94	Spring 95	Fall 95
lake trout 2-4 yr	25	25	25	25	25
lake trout 5-7 yr	25	25	25	25	25
lake trout 8-10 yr	25	25	25	25	25
coho hatchery	25			25	
coho 1 + jacks			25		25
coho 2 + adults	25	25	25	25	25
chubs 0 - 2 yr	25	25	25	25	25
chubs 4+ yr	25	25	25	25	25
alewife 60-120 mm	25	25	25	25	25
alewife »120 mm	25	25	25	25	25
smelt »100 mm	25	25	25	25	25
sculpin slimy	25	25	25	25	25
sculpin deepwater	25	25	25	25	25

Note: Lake trout were composited by age rather than length.

The same number of fish (except coho) shown in the table were repeated at Saugatuck, Port Washington, and Sturgeon Bay. The number of coho sampled was according to the table and taken across various sites each season depending on their migration location (see QA plan for Holey & Elloit, USFWS, Greenbay, WI).

1.0 Fish Processing Method

The following sample preparation procedure was originally developed for the International Joint Commission (I.J.C.) Surveillance Program. The sites, species, sizes and seasons collected and composites were modified for the Mass Balance Study.

2.0 Collection

Whole fish were collected from Lake Michigan (intact, with all body fluids and no incisions, except lake trout, which had stomachs removed), wrapped in aluminum foil, placed in 4 mil thick polyethylene bags after collection, tagged, and frozen as soon as possible on board the vessel. The information on the tag included species, size, date, location of collection and labeled for the Lake Michigan Mass Balance Study. Fish were transported to NBS/GLSC in coolers and stored frozen at about -20°C.

3.0 Aging

Prior to homogenization lake trout were first aged. To age the fish, the head of each whole fish was checked for the presence of a coded wire tag (CWT) and clipped fins to age the fish. If a CWT was detected, (CWTs are only a few mm long) with a special metal detector the first two or three cm of the fish snout was cut off and checked again with the detector to see if it contained the CWT. If not the next few cm of the snout was cut off and checked with the detector. The cut off section of the snout containing the CWT was cut in half and the half containing the CWT was cut in half again. This procedure was repeated until the tag was found or the remaining piece was less than a gram. At this point the tissue containing the CWT was placed in 10 mL solution of 15-30% NaOH for digestion. After a few hours the CWT was removed from the solution of NaOH using a small suitable teflon coated magnet and placed under a microscope. Using 5 or 10 magnification on the scope, the series of marks on the CWT were recorded. The sequence of these markings was decoded using an instruction sheet which made it possible to determine the date the fish was hatched along with other information. This date was subtracted from the date collected to determine age.

Scales were also taken from each lake trout and the fin clips were recorded. Lake trout that contained no CWT were aged by a combination of reading annual rings on the scales and fin clips. Because of the uncertainty of aging lake trout over seven years old from the scale, these age results were compared to fish in stocking records that would have the same combinations of fin clips and resulting age was base on the stocking data. If the age determined from the scale and fin clips did not match the age by the scale method we would substitute the aged lake trout in question with one of the extra lake trout collected. In cases where there were no extra fish (rare) and the age by scales and fin clips in Lake Michigan stocking records were more than two years apart the fin clips records from other Great Lakes were checked for a better match. It has been determined from tagging records that a few lake trout migrate to Lake Michigan from other Great Lakes.

4.0 Homogenization

Fish were removed from the freezer at the GLSC and allowed to thaw slowly over an 8 to 12 hour period in their sealed bags (generally overnight). Prior to homogenization, glass jars (4 oz) that were used to store subsamples were prepared by first washing in a dishwasher, then rinsed (in

sequence) with in HNO3, Millipore-filtered water, and acetone.

The contents of the polyethylene bag (fish and fluids) were weighed and recorded in the grinding log. For each species, location, and season sampled (Table 1) about 75 fish (covering three sizes or age for lake trout) were composited into about 15 samples and then ground. For a given year, site, and season lakes trout were sorted into composite samples. Depending on the number of fish in an age group available, each composite contained 2-5 fish (five when available) of the same age. Other species of the fish were sorted into five fish composite samples according to year, location, species, and size range. Each composite is put into an aluminum pan which had been cleaned with detergent and water and rinsed with deionized water. The fish were measured (millimeters) on a measuring board that was washed with detergent/water, and rinsed with distilled water. Each fish was weighed to the nearest gram and length measured to the nearest mm. The measuring board, balance, and scalpel were cleaned between each group. Homogenization equipment was washed with detergent/water, rinsed with millipore water, and then with acetone (alcohol for plastic pieces) before each sample was ground. Each composite sample was homogenized (except lake trout which were homogenized individually) and a fixed weight was sub-sampled from each lake trout for the composite and then the resulting sample was re-homogenized. Large fish such as adult lake trout and coho were homogenized using a high speed 40 qt. Hobart vertical cutter Mixer (VCM). Medium size fish were homogenized with a 12 qt. Stephan Machinery vertical cutter (UM 12) and small fish with a high speed two quart Robot Coupe (RSI241). When the large and medium size vertical cutters were used for homogenization about 1000 g of subsamples was taken and re-homogenized using the Robot Coupe cutter which obtained a finer consistency. From the final homogenized tissue about 80 g was added to each of three (depending on the amount of homogenized tissue) 4 oz jars, the lids (lined with acetone rinse aluminum foil) were screwed on, and then each jar was labeled with the identification number and the grams of tissue. The jars were boxed and then placed into the freezer (approx. -20° C) until analyzed.